

What is claimed is:

1. An amperometric sensor for the determination of glucose in aqueous media comprising:  
a sensing electrode, the sensing electrode comprising:

5 a non-conductive support member coated with an electrically conductive layer  
wherein the electrically conductive layer is further coated with a sensing electrode formulation  
comprising a first redox mediator, glucose oxidase, at least one surfactant, at least one stabilizer  
and a buffering agent to maintain a pH from about 4 to about 8;

a reference electrode comprising:

10 a non-conductive support member coated with an electrically conductive  
layer wherein the electrically conductive layer is further coated with a reference electrode  
formation comprising Ag/AgCl dispersed in a resin formation;

a passive cover electrode comprising an electrically conductive layer on a non-conductive  
support member, with the electrically conductive layer coated with a passive cover electrode

15 formulation comprising a second redox mediator, and with the passive cover electrode having an  
opening; and

non-electrically conductive spacers disposed parallel at the tops of the sensing electrode  
formulation and the reference electrode formation so that the sensing electrode and the reference  
electrode do not come into physical contact with the passive cover electrode when the latter is

20 placed over to define a reaction zone in-between the spacers;

with the electrically conducting layer of the sensing electrode and the electrically  
conductive layer of the reference electrode arrayed side-by side; and with the passive cover  
electrode placed over the side-by-side sensing electrode and reference electrode so that the active

surface of the passive cover electrode opposes with the active surfaces of the side-by-side electrodes such that the opening exposes a portion of the electrically conducting layer of the sensing electrode and a portion of the electrically conductive layer of the reference electrode that is separated by a separation within the reaction zone to receive the aqueous media.

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2. The sensor of claim 1 wherein the non-conductive support member of the sensor electrode further comprises an electrically conductive track extended from and connected to the electrically conductive layer.

10 3. The sensor of claim 2 wherein the non-conductive support member of the reference electrode further comprises an electrically conductive track extended from and connected to the electrically conductive layer.

15 4. The sensor of claim 3 wherein the electrically conductive tracks comprise an electrically conductive material selected from the group consisting of metallic silver, silver salts or mixtures thereof, conductive graphite or carbon, copper, platinum, cobalt, nickel, gold and electrically conductive polymers.

20 5. The sensor of claim 4 wherein the first redox mediator contained in the sensor electrode formulation is selected from the group consisting of ferrocyanide salts ( $\text{Fe}(\text{CN})_6^{4-}$ ),  $\text{HCN}$ ,  $\text{I}^-$ ,  $\text{Co}(\text{NH}_3)_6^{++}$ ,  $\text{Sn}^{+2}$ ,  $\text{S}^-$ ,  $\text{Tl}^{+2}$  and mixtures thereof, methyl viologen, methylene blue, thialene, iodine, dimethylferrocene (DMF) ferricinium, ferrocene monocarboxylic acid ( $\text{FCOOH}$ ), 7,7,8,8-tetracyanoquinodimethane (TCNQ), tetrathiafulvalene (TTF), nickelocene (Nc), N-

Methylacridinium ( $\text{NMA}^+$ ), tetrathiafulvalene (TTT), N-methylphenazinium ( $\text{NMP}^+$ )

hydroquinone, quinhydrone, 3,3',5,5' - tetramethylbenzidine (TMB); 3-methyl-2-

benzothiazolinone hydrazone hydrochloride and 3-dimethylaminobenzoic acid (MBTH-

DMAB); o-dianisidine; o-toluidine; sulfonated 2,4-dichloro-phenol plus 4-amino phenazone;

- 5 benzidine; 3-methyl-2-benzothiazolinone hydrazone plus 3-(dimethylamino) benzoic acid or 2-methoxy-4 allyl phenol; 4- aminoantipyrene-dimethylaniline and 4-aminoantipyrene -4-methoxynaphthol; and mixtures thereof.

6. The sensor of claim 5 wherein the surfactant is selected from the group consisting of

- 10 cholic acid, Triton X-100, polyethylene glycol, sodium lauryl sulfate, sodium lauryl sarcosinate, hydroxypropyl methylcellulose ("Methocel" 40-101 personal care grade), tetrapropylene diphenyloxide disulphonate sodium salt ("DOWFAX 2A1"), capryloamphocarboxyropinoate ("MIRALOL J2M-SF") polyoxyethylene-2-cetyl ether, Surfynol 485, MEGA-8, MEGA-10 and mixtures thereof.

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7. The sensor of claim 6 wherein the stabilizer is selected from the group consisting of gelatin, bovine serum albumin, glutamate. L-arginine, Gantrez, mannitol, gum Arabic, low viscosity polypep, methocel and mixtures thereof used separately or in combination

- 20 8. The sensor of claim 7 wherein the buffer is selected from the group consisting of citrate salts succinate salts, tris-(hydroxymethyl) aminomethane, phosphate salts, 2(N-morpholino) ethanesulfonic acid and mixtures thereof.

9. The sensor of claim 8 wherein the electrically conductive layers of the sensing electrode, the reference electrode and the passive cover electrode comprise electrically conductive carbon or graphite, copper, silver, gold, platinum, nickel, stainless steel, iron and mixtures thereof.

5 10. The sensor of claim 9 wherein the second redox mediator is the same as or different from that of the first redox mediator used in the sensing electrode.

11. The sensor of claim 1 wherein the non-conductive support member comprises a cohesive non-conductor selected from the group consisting of any non-conductive film, sheet forming  
10 polymeric material, ceramics, glass, paper, and cardboard.

12. The sensor of claim 11 wherein the sheet forming polymeric material is selected from the group consisting of polyvinyl chloride, polyester, polycarbonate, vinyl acetate copolymer, nylon, poly (1,4-butleneterephthalate), cellulose propionate, ethylene/acrylic acid copolymer,  
15 polybutadiene, polyethylene, polypropylene, polyimide, acrylic film, polyurethane, polystyrene, and polyvinyl fluoride.

13. The sensor of claim 1 wherein the spacers comprises any polymeric non-conductive material such as adhesives and double sided adhesive tape or adhesive laminating tapes.

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14. An amperometric sensor for the determination of glucose in aqueous media comprising:  
a sensing electrode, the sensing electrode comprising:

a non-conductive support member coated with an electrically conductive layer having an electrically conductive track extended therefrom wherein the electrically conductive layer is further coated with a sensing electrode formulation comprising a redox mediator, glucose oxidase, at least one surfactant, at least one stabilizer and a buffering agent to maintain a pH  
5 from about 4 to about 8;

a reference electrode comprising:

a non-conductive support member coated with an electrically conductive layer having an electrically conductive track extended therefrom wherein the electrically conductive layer is further coated with a reference electrode formation comprising Ag/AgCl  
10 dispersed in a resin formation;

a passive cover electrode comprising an electrically conductive layer on a non-conductive support member, with the electrically conductive layer coated with a passive cover electrode formulation comprising the redox mediator as used in the sensing electrode, and with the passive cover electrode having an opening; and

15 non-electrically conductive spacers disposed parallel at the tops of the sensing electrode formulation and the reference electrode formation so that the sensing electrode and the reference electrode do not come into physical contact with the passive cover electrode when the latter is placed over to define a reaction zone in-between the spacers;

with the electrically conducting layer of the sensing electrode and the electrically  
20 conductive layer of the reference electrode arrayed side-by side; and with the passive cover electrode placed over the side-by-side sensing electrode and reference electrode so that the active surface of the passive cover electrode opposes with the active surfaces of the side-by-side

electrodes such that the opening exposes only a portion of the electrically conducting layer of the sensing electrode within the reaction zone to receive the aqueous media.

15. The sensor of claim 14 wherein the non-conductive support member of the sensor  
5 electrode further comprises an electrically conductive track extended from and connected to the electrically conductive layer.

16. The sensor of claim 15 wherein the non-conductive support member of the reference  
10 electrode further comprises an electrically conductive track extended from and connected to the electrically conductive layer.

17. The sensor of claim 16 wherein the electrically conductive tracks comprise an  
electrically conductive material selected from the group consisting of metallic silver, silver salts  
or mixtures thereof, conductive graphite or carbon, copper, platinum, cobalt, nickel, gold and  
15 electrically conductive polymers.

18. The sensor of claim 17 wherein the first redox mediator contained in the sensor electrode  
formulation is selected from the group consisting of ferrocyanide salts ( $\text{Fe}(\text{CN})_6^{4-}$ ),  $\text{HCN}$ ,  $\text{I}^-$ ,  
 $\text{Co}(\text{NH}_3)_6^{++}$ ,  $\text{Sn}^{+2}$ ,  $\text{S}^{--}$ ,  $\text{Tl}^{+2}$  and mixtures thereof, methyl viologen, methylene blue, thialene,  
20 iodine, dimethylferrocene (DMF) ferricinium, ferrocene monocarboxylic acid ( $\text{FCOOH}$ ), 7,7,8,8-  
tetracyanoquinodimethane (TCNQ), tetrathiafulvalene (TTF), nickelocene (Nc), N-  
Methylacridinium ( $\text{NMA}^+$ ), tetrathiafulvalene (TTT), N-methylphenazinium ( $\text{NMP}^+$ )  
hydroquinone, quinhydrone, 3,3',5,5'-tetramethylbenzidine (TMB); 3-methyl-2-

benzothiazolinone hydrazone hydrochloride and 3-dimethylaminobenzoic acid (MBTH-DMAB); o-dianisidine; o-toluidine; sulfonated 2,4-dichloro-phenol plus 4-amino phenazone; benzidine; 3-methyl-2-benzothiazolinone hydrazone plus 3-(dimethylamino) benzoic acid or 2-methoxy-4 allyl phenol; 4- aminoantipyrene-dimethylaniline and 4-aminoantipyrene -4-methoxynaphthol; and mixtures thereof.

19. The sensor of claim 18 wherein the surfactant is selected from the group consisting of cholic acid, Triton X-100, polyethylene glycol, sodium lauryl sulfate, sodium lauryl sarcosinate, hydroxypropyl methylcellulose ("Methocel" 40-101 personal care grade), tetrapropylene diphenyloxide disulphonate sodium salt ("DOWFAX 2A1"), capryloamphocarboxypropionate ("MIRALOL J2M-SF") polyoxyethylene-2-cetyl ether, Surfynol 485, MEGA-8, MEGA-10 and mixtures thereof.

20. The sensor of claim 19 wherein the stabilizer is selected from the group consisting of gelatin, bovine serum albumin, glutamate. L-arginine, Gantrez, mannitol, gum Arabic, low viscosity polypep, methocel and mixtures thereof used separately or in combination

21. The sensor of claim 20 wherein the buffer is selected from the group consisting of citrate salts succinate salts, tris-(hydroxymethyl) aminomethane, phosphate salts, 2(N-morpholino) ethanesulfonic acid and mixtures thereof.

22. The sensor of claim 21 wherein the electrically conductive layers of the sensing electrode, the reference electrode and the passive cover electrode comprise electrically

conductive carbon or graphite, copper, silver, gold, platinum, nickel, stainless steel, iron and mixtures thereof.

23. The sensor of claim 22 wherein the second redox mediator is the same as or different  
5 from that of the first redox mediator used in the sensing electrode.

24. The sensor of claim 14 wherein the non-conductive support member comprises a cohesive non-conductor selected from the group consisting of any non-conductive film, sheet forming polymeric material, ceramics, glass, paper, and cardboard.

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25. The sensor of claim 24 wherein the sheet forming polymeric material is selected from the group consisting of polyvinyl chloride, polyester, polycarbonate, vinyl acetate copolymer, nylon, poly (1,4-butleneterephthalate), cellulose propionate, ethylene/acrylic acid copolymer, polybutadiene, polyethylene, polypropylene, polyimide, acrylic film, polyurethane, polystyrene,  
15 and polyvinyl fluoride.

26. The sensor of claim 14 wherein the spacers comprises any polymeric non-conductive material such as adhesives and double sided adhesive tape or adhesive laminating tapes.

20 27. A method for assaying a sample for determination of the concentration of glucose in an aqueous medium, comprising the steps of:

- a) providing an amperometric biosensor for glucose comprising:  
a sensing electrode, the sensing electrode comprising:



a non-conductive support member coated with an electrically conductive layer wherein the electrically conductive layer is further coated with a sensing electrode formulation comprising a first redox mediator, glucose oxidase, at least one surfactant, at least one stabilizer and a buffering agent to maintain a pH from about 4 to about 8;

5 a reference electrode comprising:

a non-conductive support member coated with an electrically conductive layer wherein the electrically conductive layer is further coated with a reference electrode formation comprising Ag/AgCl dispersed in a resin formation;

10 a passive cover electrode comprising an electrically conductive layer on a non-conductive support member, with the electrically conductive layer coated with a passive cover electrode formulation comprising a second redox mediator, and with the passive cover electrode having an opening; and

non-electrically conductive spacers disposed parallel at the tops of the sensing electrode formulation and the reference electrode formation so that the sensing electrode and the reference  
15 electrode do not come into physical contact with the passive cover electrode when the latter is placed over to define a reaction zone in-between the spacers;

with the electrically conducting layer of the sensing electrode and the electrically  
conductive layer of the reference electrode arrayed side-by side; and with the passive cover  
electrode placed over the side-by-side sensing electrode and reference electrode so that the  
20 active surface of the passive cover electrode opposes with the active surfaces of the side-by-side electrodes such that the opening exposes a portion of the electrically conducting layer of the sensing electrode and a portion of the electrically conductive layer of the reference

electrode that is separated by a separation within the reaction zone to receive the aqueous media.

b) introducing a sample into the opening of the passive cover electrode;

5 c) maintaining a potential of about -450 mV across the sensing electrode and the reference electrode;

d) measuring the current passing between the sensing electrode and the reference electrode; and

10 e) comparing the current measured to a calibration curve of the concentration of glucose versus current at the potential used in step c) to obtain the concentration of glucose in the sample.

28. The method of claim 27 wherein the non-conductive support member of the sensor electrode of step a) further comprises an electrically conductive track extended from and connected to the electrically conductive layer.

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29. The method of claim 28 wherein the non-conductive support member of the reference electrode of step a) further comprises an electrically conductive track extended from and connected to the electrically conductive layer.

20 30. The method of claim 29 wherein the electrically conductive tracks of step a) comprise an electrically conductive material selected from the group consisting of metallic silver, silver salts or mixtures thereof, conductive graphite or carbon, copper, platinum, cobalt, nickel, gold and electrically conductive polymers.

31. The method of claim 30 wherein the first redox mediator contained in the sensor electrode formulation of step a) is selected from the group consisting of ferrocyanide salts ( $\text{Fe}(\text{CN})_6^{4-}$ ),  $\text{HCN}$ ,  $\text{I}^-$ ,  $\text{Co}(\text{NH}_3)_6^{++}$ ,  $\text{Sn}^{+2}$ ,  $\text{S}^{--}$ ,  $\text{Tl}^{+2}$  and mixtures thereof, methyl viologen, methylene blue, thialene, iodine, dimethylferrocene (DMF) ferricinium, ferrocene monocarboxylic acid ( $\text{FCOOH}$ ), 7,7,8,8-tetracyanoquinodimethane (TCNQ), tetrathiafulvalene (TTF), nickelocene (Nc), N-Methylacridinium ( $\text{NMA}^+$ ), tetrathiafulvalene (TTT), N-methylphenazinium ( $\text{NMP}^+$ ) hydroquinone, quinhydrone, 3,3',5,5' - tetramethylbenzidine (TMB); 3-methyl-2-benzothiazolinone hydrazone hydrochloride and 3-dimethylaminobenzoic acid (MBTH-DMAB); o-dianisidine; o-toluidine; sulfonated 2,4-dichloro-phenol plus 4-amino phenazone; benzidine; 3-methyl-2-benzothiazolinone hydrazone plus 3-(dimethylamino) benzoic acid or 2-methoxy-4 allyl phenol; 4- aminoantipyrene-dimethylaniline and 4- aminoantipyrene -4-methoxynaphthol; and mixtures thereof.
32. The method of claim 31 wherein the surfactant of step a) is selected from the group consisting of cholic acid, Triton X-100, polyethylene glycol, sodium lauryl sulfate, sodium lauryl sarcosinate, hydroxypropyl methylcellulose ("Methocel" 40-101 personal care grade), tetrapropylene diphenyloxide disulphonate sodium salt ("DOWFAX 2A1"), capryloamplhocarboxyropinoate ("MIRALOL J2M-SF") polyoxyethylene-2-cetyl ether, Surfynol 485, MEGA-8, MEGA-10 and mixtures thereof.

33. The method of claim 32 wherein the stabilizer of step a) is selected from the group consisting of gelatin, bovine serum albumin, glutamate, L-arginine, Gantrez, mannitol, gum Arabic, low viscosity polypep, methocel and mixtures thereof used separately or in combination

5 34. The method of claim 33 wherein the buffer of step a) is selected from the group consisting of citrate salts succinate salts, tris-(hydroxymethyl) aminomethane, phosphate salts, 2(N-morpholino) ethanesulfonic acid and mixtures thereof.

35. The method of claim 34 wherein the electrically conductive layers of the sensing  
10 electrode, the reference electrode and the passive cover electrode of step a) comprise electrically conductive carbon or graphite, copper, silver, gold, platinum, nickel, stainless steel, iron and mixtures thereof.

36. The method of claim 35 wherein the second redox mediator of step a) is the same as or  
15 different from that of the first redox mediator used in the sensing electrode.

37. The method of claim 36 wherein the non-conductive support member of step a) comprises a cohesive non-conductor selected from the group consisting of any non-conductive film, sheet forming polymeric material, ceramics, glass, paper, and cardboard.

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38. The method of claim 37 wherein the sheet forming polymeric material is selected from the group consisting of polyvinyl chloride, polyester, polycarbonate, vinyl acetate copolymer, nylon, poly (1,4-butleneterephthalate), cellulose propionate, ethylene/acrylic acid copolymer,

polybutadiene, polyethylene, polypropylene, polyimide, acrylic film, polyurethane, polystyrene, and polyvinyl fluoride.

39. The method of claim 38 wherein the spacers of step a) comprises any polymeric non-  
5 conductive material such as adhesives and double sided adhesive tape or adhesive laminating tapes.

40. A method for assaying a sample for determination of the concentration of glucose in an aqueous medium, comprising the steps of:

10 a) providing an amperometric biosensor for glucose comprising:

a sensing electrode, the sensing electrode comprising:

a non-conductive support member coated with an electrically conductive layer wherein the electrically conductive layer is further coated with a sensing electrode formulation comprising a first redox mediator, glucose oxidase, at least one surfactant, at least one stabilizer  
15 and a buffering agent to maintain a pH from about 4 to about 8;

a reference electrode comprising:

a non-conductive support member coated with an electrically conductive layer wherein the electrically conductive layer is further coated with a reference electrode formation comprising Ag/AgCl dispersed in a resin formation;

20 a passive cover electrode comprising an electrically conductive layer on a non-conductive support member, with the electrically conductive layer coated with a passive cover electrode formulation comprising a second redox mediator, and with the passive cover electrode having an opening; and

non-electrically conductive spacers disposed parallel at the tops of the sensing electrode formulation and the reference electrode formation so that the sensing electrode and the reference electrode do not come into physical contact with the passive cover electrode when the latter is placed over to define a reaction zone in-between the spacers;

5 with the electrically conducting layer of the sensing electrode and the electrically conductive layer of the reference electrode arrayed side-by side; and with the passive cover electrode placed over the side-by-side sensing electrode and reference electrode so that the active surface of the passive cover electrode opposes with the active surfaces of the side-by-side electrodes such that the opening exposes only a portion of the electrically conducting layer of the sensing electrode  
10 within the reaction zone to receive the aqueous media;

b) introducing a sample into the opening of the passive cover electrode;

c) maintaining a potential of about -450 mV across the sensing electrode and the reference electrode;

d) measuring the current passing between the sensing electrode and the reference electrode;

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e) comparing the current measured to a calibration curve of the concentration of glucose versus current at the potential used in step c) to obtain the concentration of glucose in the sample.

20 41. The method of claim 40 wherein the non-conductive support member of the sensor electrode of step a) further comprises an electrically conductive track extended from and connected to the electrically conductive layer.

42. The method of claim 41 wherein the non-conductive support member of the reference electrode of step a) further comprises an electrically conductive track extended from and connected to the electrically conductive layer.

5 43. The method of claim 42 wherein the electrically conductive tracks of step a) comprise an electrically conductive material selected from the group consisting of metallic silver, silver salts or mixtures thereof, conductive graphite or carbon, copper, platinum, cobalt, nickel, gold and electrically conductive polymers.

10 44. The method of claim 43 wherein the first redox mediator contained in the sensor electrode formulation of step a) is selected from the group consisting of ferrocyanide salts ( $\text{Fe}(\text{CN})_6^{4-}$ ),  $\text{HCN}$ ,  $\text{I}^-$ ,  $\text{Co}(\text{NH}_3)_6^{++}$ ,  $\text{Sn}^{+2}$ ,  $\text{S}^{--}$ ,  $\text{Tl}^{+2}$  and mixtures thereof, methyl viologen, methylene blue, thialene, iodine, dimethylferrocene (DMF) ferricinium, ferrocene monocarboxylic acid ( $\text{FCOOH}$ ), 7,7,8,8-tetracyanoquinodimethane (TCNQ), tetrathiafulvalene (TTF), nickelocene (Nc), N-Methylacridinium ( $\text{NMA}^+$ ), tetrathiafulvalene (TTT), N-methylphenazinium ( $\text{NMP}^+$ ) hydroquinone, quinhydrone, 3,3',5,5'-tetramethylbenzidine (TMB); 3-methyl-2-benzothiazolinone hydrazone hydrochloride and 3-dimethylaminobenzoic acid (MBTH-DMAB); o-dianisidine; o-toluidine; sulfonated 2,4-dichloro-phenol plus 4-amino phenazone; benzidine; 3-methyl-2-benzothiazolinone hydrazone plus 3-(dimethylamino) benzoic acid or 2-methoxy-4 allyl phenol; 4- aminoantipyrene-dimethylaniline and 4- aminoantipyrene -4-methoxynaphthol; and mixtures thereof.

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45. The method of claim 44 wherein the surfactant of step a) is selected from the group consisting of cholic acid, Triton X-100, polyethylene glycol, sodium lauryl sulfate, sodium lauryl sarcosinate, hydroxypropyl methylcellulose ("Methocel" 40-101 personal care grade), tetrapropylene diphenyloxide disulphonate sodium salt ("DOWFAX 2A1"),  
5 capryloamphocarboxypinoate ("MIRALOL J2M-SF") polyoxyethylene-2-cetyl ether, Surfynol 485, MEGA-8, MEGA-10 and mixtures thereof.

46. The method of claim 45 wherein the stabilizer of step a) is selected from the group consisting of gelatin, bovine serum albumin, glutamate, L-arginine, Gantrez, mannitol, gum  
10 Arabic, low viscosity polypep, methocel and mixtures thereof used separately or in combination

47. The method of claim 46 wherein the buffer of step a) is selected from the group consisting of citrate salts succinate salts, tris-(hydroxymethyl) aminomethane, phosphate salts, 2(N-morpholino) ethanesulfonic acid and mixtures thereof.

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48. The method of claim 47 wherein the electrically conductive layers of the sensing electrode, the reference electrode and the passive cover electrode of step a) comprise electrically conductive carbon or graphite, copper, silver, gold, platinum, nickel, stainless steel, iron and mixtures thereof.

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49. The method of claim 48 wherein the second redox mediator of step a) is the same as or different from that of the first redox mediator used in the sensing electrode.



50. The method of claim 49 wherein the non-conductive support member of step a) comprises a cohesive non-conductor selected from the group consisting of any non-conductive film, sheet forming polymeric material, ceramics, glass, paper, and cardboard.

5 51. The method of claim 50 wherein the sheet forming polymeric material is selected from the group consisting of polyvinyl chloride, polyester, polycarbonate, vinyl acetate copolymer, nylon, poly (1,4-butleneterephthalate), cellulose propionate, ethylene/acrylic acid copolymer, polybutadiene, polyethylene, polypropylene, polyimide, acrylic film, polyurethane, polystyrene, and polyvinyl fluoride.

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52. The method of claim 51 wherein the spacers of step a) comprises any polymeric non-conductive material such as adhesives and double sided adhesive tape or adhesive laminating tapes.